

Amendment

Amendments to the Specification:

Please replace the paragraphs beginning at page 6, line 17 through page 9, line 1, with the following rewritten paragraphs:

Figure 1. Identification of a decapping activity in HeLa cytoplasmic extracts. ~~Panel A~~ Figure 1A. SVARE-A0 RNA, radiolabeled exclusively at the alpha-phosphate of the 5' cap structure, was incubated in standard decapping conditions using no extract, *S. cerevisiae* whole cell extract (yeast lane), or HeLa S100 cytoplasmic extract. The ^{7me}GDP product of the decapping reaction was resolved by thin layer chromatography on PEI cellulose sheets developed using 0.45 M ammonium sulfate. The identification of radioactive spots was determined using markers that were visualized by UV shadowing. ~~Panel B~~ Figure 1B. GemARE-A60 RNA, radiolabeled exclusively at the alpha-phosphate of the 5' cap structure, was incubated in the absence of extract (no extract lane) or in the presence of three independently prepared S100 cytoplasmic extracts from HeLa cells (A, B, or C) either in the absence (-lanes) or presence of 20 micromolar cap analog (+ lanes). The ^{7me}GDP product of the decapping reaction was resolved by chromatography on PEI cellulose sheets developed using 0.45 M ammonium sulfate.

Figure 2. Methylated cap analog specifically activates decapping in HeLa cytoplasmic extracts by sequestering cap-binding proteins. ~~Panel A~~ Figure 2A. The

indicated amounts of ^7me GpppG or GpppG were incubated with HeLa S100 extracts and decapping assays were performed using cap-labeled GemARE-A60 RNA. The products of decapping were analyzed on PEI-cellulose sheets. The arrowhead indicates ^7me GDP. ~~Panel-B~~ Figure 2B. The indicated amounts of ^7me GpppG or GpppG were incubated in HeLa S100 extracts using cap-labeled GemARE-A60 RNA under decapping conditions. After 5 min., UV cross-linking was performed, mixtures were treated with ribonuclease, and proteins radiolabeled through cross-linking to cap-labeled RNA oligomers were analyzed by electrophoresis on a 15% acrylamide gel containing SDS. The position of eIF4E is indicated by the arrowhead. ~~Panel-C~~ Figure 2C. The indicated amounts of ^7me GpppG or GpppG were incubated in HeLa S100 extracts using cap-labeled GemARE-A60 RNA under decapping conditions. After 5 min., UV cross-linking was performed, mixtures were treated with ribonuclease, DAN/PARN proteins radiolabeled through cross-linking to cap-labeled RNA oligomers were immunoprecipitated and analyzed by electrophoresis on a 10% acrylamide gel containing SDS. The position of DAN/PARN is indicated by the arrowhead. ~~Panel-D~~ Figure 2D. The indicated amounts of ^7me GpppG or GpppG were incubated in HeLa S100 extracts using cap-labeled GemARE-A0 RNA under decapping conditions. The top panel shows UV cross-linking analysis or total protein as described in Figure 2B to identify eIF4E. The middle panel shows UV cross-linking/immunoprecipitation analysis as described in Figure 2C to identify DAN/PARN. The bottom panel shows the products of a decapping assay that were analyzed by thin layer chromatography as described in Figure 2A.

Figure 3. The presence of a poly(A) tail represses decapping of three independent RNA substrates. Equimolar amounts of three independent, cap-labeled RNA substrates that either lacked a poly(A) tail (GemARE-A0 (Figure 3A), SVARE-A0 (Figure 3B), and GM-CSF-A0 (Figure 3C)) or contained 60 adenylate residues at their 3' end (GemARE-A60 (Figure 3A), SVARE-A60 (Figure 3B) and GM-CSFT-A60 (Figure 3C)) were incubated in the *in vitro* decapping assay in the presence of cap analog. Aliquots were removed at the indicated time points and reaction products were analyzed by thin layer chromatography on PEI cellulose sheets.

Figure 4. The addition of poly(A) competitor RNA specifically activated decapping of polyadenylated RNA substrates. ~~Panel A-Figure 4A~~. Cap-labeled GemARE-A60 RNA, which contained 60 adenylate residues at its 3' end, was incubated in the *in vitro* decapping system for 30 minutes in the presence of cap analog and the indicated amount of cold poly (A) competitor RNA. Reaction products were analyzed by thin layer chromatography on PEI cellulose sheets. In the lane marked GemARE-A0, GemARE-A0 RNA (that lacks a poly(A) tail) was incubated in the *in vitro* decapping assay in the absence of poly(A) RNA competitor. ~~Panel B-Figure 4B~~. Cap-labeled GemARE-A60 RNA, was incubated in the *in vitro* decapping system for 30 minutes in the presence of cap analog and the indicated amount of cold poly(A) or poly(C) competitor RNAs. Reaction products were analyzed by thin layer chromatography on PEI cellulose sheets. Input RNA was run in the lane designated input. ~~Panel C-Figure 4C~~. Cap-labeled GemARE-A0 RNA, which lacked a poly(A) tail, was incubated in the *in*

vitro decapping system for 30 minutes in the presence of cap analog and the indicated amount of cold poly(A) competitor RNA. Reaction products were analyzed by thin layer chromatography on PEI cellulose sheets. For all three ~~panels~~ Figures 4A, 4B and 4C, the position of ^{7me}GDP is indicated by the arrowhead.

Figure 5. The presence of an AU-rich element significantly stimulates the efficiency of decapping. ~~Panel A~~ Figure 5A, and ~~Panel B~~ Figure 5B. A matched pair of cap-labeled RNA substrates that either lacked (Gem-A0 or SV-A0) or contained the TNF-alpha AU-rich element (GemARE-A0 or SVARE-A0) were incubated in the *in vitro* decapping system in the presence of cap analog for the indicated amount of time. Reaction products were analyzed by thin layer chromatography on PEI cellulose sheets. ~~Panel C~~ Figure 5C. A matched pair of cap-labeled RNA substrates that either lacked (GM-CSF(-ARE)) or contained the GM-CSF AU-rich element (GM-CSF(+ARE)) were incubated in the *in vitro* decapping system in the presence of cap analog for the indicated amount of time. Reaction products were analyzed by thin layer chromatography on PEI cellulose sheets. For all three ~~panels~~ Figures 5A, 5B and 5C, the position of ^{7me}GDP is indicated by an arrowhead.

RESPONSE UNDER 37 C.F.R. § 1.116

EXPEDITED PROCEDURE – Art Unit 1636

Attorney Docket No. 54569.8009.US01

Amendments to the Drawings:

The attached replacement sheets of drawings include changes made to Fig. 1-9.

Please replace the original sheets of drawings with the attached replacement sheets.

Attachment: Replacement Sheets of Drawings.